Detection of some virulence genes of *Pseudomonas aeruginosa* isolated from meat at Mosul city

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**Abstract**

Meat is a rich source of protein for humans. *Pseudomonas* is one of the meat spoilage microorganisms, as it is highly pathogenic and affects the health of consumers and meat handlers. The current study detected the presence of some virulence genes in *Pseudomonas aeruginosa* strains isolated from meat in Mosul city, including *ToxA*, *ExoS*, *OprL*, and *PlcH* virulence genes in 21 isolates of *P. aeruginosa* using PCR. Results revealed the presence of *ToxA*, *OprL*, and *PlcH* genes at 57.14%, 38.09%, and 71.42%, respectively, while all the isolates of *P. aeruginosa* were negative for the presence of the *ExoS* gene. The prevalence of *ToxA* and *PlcH* genes was higher in beef meat compared to mutton and chicken meat, while the *OprL* gene was not detected in mutton. These results indicated that some strains of *P. aeruginosa* are pathogenic to meat handlers and consumers; following food safety practices must be applied in the meat production chain to prevent meat contamination with pathogenic bacteria.

**Introduction**

In Mosul, people eat a variety of meats displayed in markets, such as beef, mutton, and chicken. Improper hygienic conditions in handling and preserving meats predisposed them to spoilage because meat is a suitable medium to growth of different types of microorganisms (1,2). Proliferations of these bacterial groups lead to meat deterioration (3,4) especially psychrotrophic bacteria which can arise in meats stored aerobically at low temperature (5-7). *Pseudomonas aeruginosa* is one of the most common specific spoilage microorganisms in meat and meat products which make them unpalatable as a result of discoloration, off-odor, off-flavor, and slime production (8-10). *Pseudomonas aeruginosa* described as an opportunistic pathogen associated with health hazards (11-13). Along with skin infections, markedly contributed to a wide range of infections in many organs, including the respiratory, urinary, and gastrointestinal tracts due the presence of cell mediated virulence factors like flagella, pilli, lipopolysaccharides, exoproteases, exotoxins and phospholipase C which are frequently implicated in bacterial motility and colonization (14,15). In addition, *P. aeruginosa* has the ability to form biofilms which increased its resistance to antimicrobial agents (16,17). Exotoxin A is one of these virulence elements that is crucial for tissue lysis and bacterial invasion. It employs a pilus-like apparatus to release proteins into the extracellular environment, including lipase, phospholipase, alkaline phosphatase, and protease (18,19). Lecithin and lipids that contribute to tissue invasion are destroyed by the hemolysin phospholipase H (plcH) enzymes. Additionally, *P. aeruginosa* produces elastase B (lasB), a key player in the acute infection, and exoenzyme S (*ExoS*), a cytotoxin that causes harm to a variety of host cells. It’s an ADP ribosyl transferase that is secreted by the type III secretion system straight into the cytoplasm of epithelial cells (20). *OprL*, gene is also used as a marker to rapid identification of virulent *P. aeruginosa* strains (21).
Available studies about the virulence factors of *P. aeruginosa* in meat are not sufficient. The current study aimed to detect some virulence factors genes in *P. aeruginosa* isolated from beef, mutton, and chicken meat displayed in Mosul city retails using PCR technique.

**Materials and methods**

**Ethical approve**

The scientific committee of the veterinary public health department approved this research on the twelfth session at 20/June/2021.

**Samples**

The study included twenty-one isolates of *P. aeruginosa* obtained from 150 samples of fresh beef, mutton and chicken meat displayed in Mosul city retails in our previous study, these isolates were distributed as 7 isolates from beef meat, 3 isolates from mutton, and 11 isolates from chicken meat, the isolates were previously confirmed using molecular detection depending on *rpoB* gene using polymerase chain reactions (22,23).

**Isolation and identifications**

*P. aeruginosa* isolates from meat was inoculated on brain heart infusion broth media (Himedia/Indian) and incubated at 37°C for 24hrs. Followed by inoculation of growing bacteria on cetrimide agar (Neogen/USA) then incubated overnight at 37°C to select the pure isolates of *P. aeruginosa* (24).

**DNA extraction**

Genomic DNA of *P. aeruginosa*. was extracted using bacterial DNA preparation kit (Gena bioscience/Germany) following the manufacturer’s instructions (23).

**Polymerase chain reaction (PCR)**

Molecular detection of *P. aeruginosa* virulence genes was done using specific primers targeting some virulence genes including *ToxA*, *ExoS*, *OprL* and *PleH*. Each virulence genes were amplified using specific primer sequences mentioned in table 1, and provided by (Macrogen/Korea). The PCR master mix reaction prepared according to kit instructions (GeNet Bio, Korea) in 20 μl total volume by adding 2 μl of purified genomic DNA, 10 of Taq Premix (2X) and 1 μl of each forward and reverse primer 10 pmol /μl then completing the PCR premix tube by PCR grade water into 20 μl and briefly mixed by. The reaction was performed in a thermocycler (BioRad, USA) for amplification by applied the following thermocycler conditions; initial denaturation temperature of 95°C for 5 min; followed by 35 cycles at denaturation 95°C for 45s, annealing for 45 s at 55°C for each *ToxA* and *OprL* gene while the annealing of *ExoS* and *PleH* for 45s. At 60°C followed by extension 72°C for 1min and then final extension at 72°C for 10 min. The PCR products were examined by electrophoresis in a 1.5% agarose gel (BioRad, USA), stained with 3 μl prime safe dye (GeNet Bio, Korea), 4 μl of DNA ladder, 100 bp (GeNet Bio, Korea) depended as a molecular weight standard and the gel viewed using Gel doc Ez system (BioRad, USA) to identify the specific bands.

**Statistical analysis**

Chi-square test (χ²) was used to compare the prevalence of virulence factor genes among the isolates of *P. aeruginosa* strains and meat type (beef, mutton, and chicken) using IBM/SPSS/Statistics/version22, USA. A value of P<0.05 was considered statistically significant.

**Results**

Results highlighted the prevalence of some virulence factors genes of *P. aeruginosa*. strains isolated from meat. The *ToxA* gene was detected in 57.14% (12/21) of isolates. The *OprL* gene was detected in 38.09% (8/21) of isolates. Also, *PleH* gene was present in 71.42% (15/21) of *P. aeruginosa* isolates. While all the isolates were negative for the exotoxin encoding S (*ExoS*) gene. The differences between the *PleH*, *OprL*, and *ToxA* genes were statistically

**Table 1: primers sequence for some *P. aeruginosa*. virulence genes**

<table>
<thead>
<tr>
<th>Primers</th>
<th>Primer’s sequence</th>
<th>°C</th>
<th>Size (bp)</th>
<th>Genes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ExoS</em>-F</td>
<td>GCGAGGTCAGCAGATATCG</td>
<td>60</td>
<td>118</td>
<td><em>ExoS</em></td>
<td>(25)</td>
</tr>
<tr>
<td><em>ExoS</em>-R</td>
<td>TCCGGCCTCACGTGGATGC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>OprL</em>-F</td>
<td>ATGGAAATGCGTAAATTGCGG</td>
<td>55</td>
<td>504</td>
<td><em>oprL</em></td>
<td>(26)</td>
</tr>
<tr>
<td><em>OprL</em>-R</td>
<td>CTTCTTCAGCTCGACGACGAG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>ToxA</em>-F</td>
<td>GACACGCCTCACGATTCACAGAC</td>
<td>55</td>
<td>396</td>
<td><em>ToxA</em></td>
<td>(27)</td>
</tr>
<tr>
<td><em>ToxA</em>-R</td>
<td>CGCTGCGGCGTCGCTCCAGCG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>PleH</em>-F</td>
<td>GAA GCC ATG GCC TAC TTC AA</td>
<td>60</td>
<td>307</td>
<td><em>pleH</em></td>
<td>(28)</td>
</tr>
<tr>
<td><em>PleH</em>-R</td>
<td>AGA GTG ACG AGG AGC GGTAG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The differences between the *PleH*, *OprL*, and *ToxA* genes were statistically

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significant at P<0.05. The prevalence of ToxA and PlcH genes was higher in beef meat 33.33% to each of them compared to mutton and chickens’ meat while the prevalence of OprL genes was absent in mutton (Figures 1-5).

Figure 1: Prevalence of some virulence genes among P. aeruginosa. isolates from all types of meat.

Figure 2: Prevalence of some virulence genes among P. aeruginosa. isolates from beef, mutton and chicken meat.

Figure 3: Electrophoretic profile illustrate Lanes M, DNA marker; lane 1-5, ToxA gene of P. aeruginosa at 396 bp product size, lane 6 negative control.

Figure 4: Electrophoretic profile illustrate Lanes M, DNA marker; lane 1-5, OprL gene of P. aeruginosa at 504 bp product size, lane 6 negative control.

Figure 5: Electrophoretic profile illustrate Lanes M, DNA marker; lane 1-4, PlcH gene of P. aeruginosa at 307 bp product size, lane 5 negative control.

Discussion

P. aeruginosa from animal sources involved in many tissues destruction in human beings indicated their pathogenicity which is associated with the role of some virulence factors causing serious infections (29). Virulence factors of P. aeruginosa participate with an important role in the presence and growth of these microorganisms on the surfaces and invasion of tissues with the assistance of Pilis,
fimbria and polysaccharides which considered as a predisposing factor for infections (30,31). The prevalence of ToxA virulence genes in P. aeruginosa isolates from meat was relatively high while these virulence genes were absent in P. aeruginosa isolated from frozen meat (32). The prevalence of oprL genes in P. aeruginosa isolates from meat were 38.09% which disagreements with Tartol and El-Enaeey (32) who referred to absence of these genes in frozen meat. Also, the prevalence of PlcH genes in P. aeruginosa isolates were 71.42% indicating that the isolates were capable of secreting phospholipase C and hemolytic exoenzyme which may involve in pulmonary infections (33) this percentage is relatively similar to the presence of PlcH genes in pseudomonas isolated from beef (34). The high prevalence of this gene indicated its importance in the pathogenicity of these microorganisms and its ability to induce infections with high potential risk to consumer (35,36) Meanwhile the distribution of ExoS genes in P. aeruginosa isolates from meat are negative these results were disagreed with Shahat et al. (37) who referred to the presence of these genes at 71.42% of P. aeruginosa isolates in broilers. The detection of some virulence factors of P. aeruginosa may be useful in early detections of these microorganisms in meats (38). The genetic variations in P. aeruginosa isolates from different animal sources play an important role in determination of their virulence according to source of isolates. Further studies are required to exhibit the association of P. aeruginosa virulence factors with public health especially those associated with serious respiratory and urinary infections.

Conclusion

Detection of some virulence factors genes in P. aeruginosa isolated from meat indicated the high level of pathogenicity of this microorganism which may affect the consumer health. The high prevalence of these virulence genes requires to apply hygienic conditions during the handling of meat and meat products as well as through their displaying in retail.

Acknowledgments

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Conflict of interest

There are no conflicts of interest declared by the authors.

References

الکشف عن بعض جينات الضراوة في الزوائف الزنجارية المعزولة من اللحوم في مدينة الموصل

إبراهيم محمد طاهر جوهر والمهي تي حسن

Summary

Aim: This study was conducted to detect the pathogenicity genes among Pseudomonas aeruginosa isolated from chicken embryos and broilers with regard to disinfectant resistance.

Materials and Methods: This study was conducted on a total of 70 chicken embryos and broilers from the College of Veterinary Medicine, University of Mosul. The samples were collected from the period of 2015-2020. The isolation and identification of Pseudomonas aeruginosa were done using routine steps. The pathogenicity genes were amplified using specific primers for ExoS, ToxA, and PlcH. The results showed that 12% of the isolates carried one or more of the pathogenicity genes.

Results: The results showed that 12% of the isolates carried one or more of the pathogenicity genes.

Conclusion: The results indicated that Pseudomonas aeruginosa is a common pathogen in chicken embryos and broilers in the Mosul area. The presence of pathogenicity genes in some isolates indicates a potential risk for the health of livestock and humans. The findings of this study highlight the importance of implementing effective disinfection strategies to control the spread of Pseudomonas aeruginosa.